orientation. This means that for fascin bundles to form the actin filaments must occupy a parallel orientation before the structure can be stabilized. In the case of alpha-actinin structures, crosslinks could form at any point during the process of structure assembly. We have shown that crosslinking proteins recognize specific orientations of actin filaments, which places constraints on how cytoskeletal structures assemble and organize.

803-Pos

A Structural and Biochemical Study of the Interaction Between Actin and the Mammalian Formin FRL2

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Formins are a class of proteins that influence the rate of actin filament nucleation and elongation. Mammals possess 15 formin isoforms, providing a myriad of possibilities for regulating actin-based structures in cells. The dimeric formin homology 2 (FH2) domain is capable of accelerating the nucleation rate of new actin filaments and subsequently influences filament elongation via direct interaction with the barbed end of an actin filament. The FH2 domain moves processively with the barbed end as the filament elongates. A subset of formins, including FRL2, can also bundle filaments. Our goal in this study is to examine FRL2's interaction with actin in detail. A portion of FRL2 containing the FH2 domain forms a stable interaction with tetramethylrhodamine-malamide labeled actin (TMR-actin) or Latrunculin B-bound actin, two forms of actin that are unable to polymerize. The actin/FRL2 complex is mono-disperse, as judged by analytical ultracentrifugation and gel filtration. FH2 domain-containing constructs from three other mammalian formins (mDia1, mDia2, and INF2) do not show an equivalent interaction. A mutation in the FH2 domain of FRL2 that prevents barbed-end binding also prevents interaction with TMR-actin, suggesting that the interaction interface is similar to that of FRL2 with the filament barbed end. These properties make the actin/FRL2 complex an ideal system for a structural study of actin/formin interactions. Both biochemical and structural experiments are being carried out with FRL2 constructs and both LatB treated and TMR labeled actin in order to characterize the details of their binding interactions.

804-Pos

The Effect of Heavy Meromyosin on the Flexibility of Formin-Bound Actin Filaments

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Formins are conservative proteins with important roles in the regulation of the microfilamental system in eukaryotic cells. They have several domains including FH1, FH2, GPB and DAD domains. In the interaction between actin and formin the FH2 domain plays a key role. This domain builds antiparallel dimers with the help of the 'linker region' between FH1 and FH2 domains. The 'mammalian Diaphanous-related 1′ constitutes one of the subfamilies of the formins. It was shown that formins could make the actin filaments more flexible (Bugyi et al. 2006), and another actin binding protein, tropomyosin reduces this effect (Ujfalusi et al. 2008). In our work we investigated whether the flexibility of the filaments could be restored by the binding of the common binding partner of actin, myosin. Skeletal muscle and non-muscle 2B isoforms of HMM were used. Temperature dependent Förster-type resonance energy transfer (FRET) and fluorescence anisotropy decay experiments showed that the formin (mDia1-FH2) induced an increase in the flexibility of actin filaments, which was reversed by the binding of heavy meromyosin HMM. Our previous measurements showed that tropomyosin had a similar stabilizing effect on the formin-bound actin filaments. These observations together indicated that actin-binding proteins played a central role in the molecular mechanisms that regulate the dynamic properties of the actin filaments. This mechanism provides the opportunity for the formin-induced actin filaments to become similar to those that are polymerized in the absence of formins.

805-Pos

The Calponin Regulatory Region is Intrinsically Unstructured: Novel Insight into Actin-Calponin and Calmodulin-Calponin Interfaces using Nmr Spectroscopy

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h1-calponin is an actin and calmodulin binding protein. Previous studies established that the recombinant calponin fragment 131-228 binding to actin involves two distinct sites. The first actin binding site was attributed to amino acids Ala145-Ile163 while amino acids Lys172-His187 formed the second actin binding site. Here we have used nuclear magnetic resonance spectroscopy to investigate the structure of this functionally important region of calponin and map its interaction with actin and calmodulin at amino acid resolution. Our data indicates that the free calponin peptide is largely unstructured in solution, although 4 short amino acid stretches corresponding to residues 140-146, 159-165, 189-195 and 199-205 display the propensity to form α-helices. Our data identified the amino acids involved in actin binding within the first actin binding site and demonstrated for the first time that the N-terminal flanking region of Lys137-Tyr144 is an integral part of this actin binding site. We have further delineated the second actin binding site to amino acids Thr180-Asp190. Binding to calmodulin extends beyond the previously identified minimal sequence of 153-163 and includes most amino acids within the stretch 143-165. In addition we found that calmodulin induces chemical shift perturbations of amino acids 188-190 demonstrating for the first time an effect of Ca^{2+}-calmodulin on this region. The spatial relationship of the actin and calmodulin contacts within the regulatory region of calponin provides a structural framework for understanding the Ca^{2+} dependent regulation of the actin-calponin interaction by calmodulin.

806-Pos

Evidence from the Laser Trap for Two Closed States of Tropomyosin

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The position of tropomyosin (Tm) on the thin filament is often described by a three state model: 1) blocked, Ca^{2+} is absent and steric hindrance by Tm blocks myosin from binding to actin, 2) closed, Ca^{2+} binds to troponin C which unlocks Tm and partially unblocks myosin binding, and 3) open, initial myosin binding shifts Tm further, exposing downstream myosin binding sites and cooperatively activating the thin filament (McKillop and Geeves, 1993). We previously showed that Tm phosphorylation enhances force production by myosin - an effect independent of steric hindrance and thus not predicted by the current three-state model. We therefore tested the hypothesis that Tm phosphorylation affects the on-rate of single actin-myosin bonds. Heavy meromyosin (HMM) was adsorbed to immobilized, nitrocellulose-coated pedestals, and biotinylated actin filaments with natively phosphorylated or dephosphorylated Tm were coupled to streptavidin-coated beads. Using a laser trap, we measured the time necessary for the first bond to form (1/on-rate) between actin and rigor HMM. Measurements were repeated in the presence 10-20 nM N-ethylmaleimide modified myosin-S1 to force Tm from the closed to the open state. Actin-myosin on-rates were increased by Tm, but only in the closed state. Phosphorylation of Tm enhanced this effect. However, the frequency of actin-HMM bond formation was reduced in the closed state in the presence of Tm. Together, these data suggest that there may be at least two closed states of Tm in equilibrium with one another. In the first, Tm hinders myosin binding resulting in a relatively low on-rate, while in the second Tm becomes a ''guide'' to myosin binding and accelerates the on-rate.

807-Pos

Precise Modulation of Tropomyosin Polymer Length is Crucial for its Association with Actin and Ability to Regulate Myosin Function

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Tropomyosin (Tm) is an evolutionarily conserved dimeric α-helical coiled-coil protein, which interacts end to end to form polymers capable of associating with and stabilising actin-filaments and thereby regulate myosin function. The fission yeast Schizosaccharomyces pombe possesses a single Tm, known as Cdc8. Cdc8 is an essential protein, which can be acetylated on its amino terminal methionine in vivo. This acetylation increases the affinity of Cdc8 for actin, and also enhances its ability to regulate myosin function (J. Cell Sci. 120: 1635-1645). We have recently undertaken an extensive analysis on the physical properties of acetylated and unacetylated Cdc8 together with a series of novel amino terminal Cdc8 mutants, in an attempt to explore the effect acetylation has upon the regulatory function of the Cdc8 protein. By correlating the stability of each protein and its propensity to form polymers with its ability to associate with actin and regulate myosin, it has been possible to establish that precise modulation of Tm-polymer length is crucial for its function. Cdc8 mutants capable of forming Tm-polymers significantly longer than the wild-type protein had a reduced affinity for actin, and in contrast to both wild type forms of the protein were unable to regulate myosin. The longer Tm-polymers are unable to efficiently coil around the already formed actin filament, and together our data are consistent with a mechanism by which acetylation regulates the formation of short Tm-polymers (normally up of ~2 Cdc8 dimers) which associate with actin-filaments and are subsequently stabilised by electrostatic interactions with actin.